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EVALUATION OF M15/M18 ENZYME DETECTOR TICKET SYSTEM WITH LOW CONCENTRATIONS OF GB

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Edgewood Arsenal
Aberdeen Proving Ground, Maryland

June 1974

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Chemical agent detector kits

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Detection	Enzyme	Substrate
Nerve agents	Cholinesterase	Impaction
Detector kits	Static exposure	Pullthrough
GB	Buffer	Diffusion

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The objective of this investigation was to evaluate the enzyme ticket system in the M15A2 and M18A2 detector kits, using various types of exposure, for the detection of low concentrations of GB. The tickets met the requirements for the chemical agent detector kit (0.03 ± 0.02 μg/l in less than 10 min). They were able to detect 0.02 μg/l by static exposure for 8 min. Concentrations as low as 0.003 μg/l could also be detected by static exposure for 90 min but required periodic rewetting. Using a piston-type hand pump and a special impacting head, it was possible to detect 0.003 μg/l with a minimum of 40 strokes (approximately 2 min of sampling).

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PREFACE

The work described in this report was authorized under Task 1B663721D60108, Chemical Agent Detector Kits. This work was started in April and completed in November 1971.

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EVALUATION OF M15/M18 ENZYME DETECTOR TICKET SYSTEM WITH LOW CONCENTRATIONS OF GB

I. INTRODUCTION.

The detector ticket for anticholinesterase agents was developed as a part of the M15A1 (E27R6) and M18A1 (E28R2) detector kits.[1] The ticket consists of a polypropylene piece (1 inch wide, 2 inches long, 1/16 inch thick), round at one end, square at the other, and hinged in the center. Each half of the ticket contains a glass fiber disc (7/16 inch in diameter) impregnated with horse serum cholinesterase. It is packaged in an envelope of mylar/polyethylene and is used by first wetting the enzyme on the square end with buffer solution (pH 8.0), exposing to suspect air, and adding substrate (2,6-dichloroindophenyl acetate in ligroin) from a dispenser. The same procedure is followed using the round (control) end of the ticket except that this end is not exposed to suspect contamination. Both ends of the ticket are then examined for color change. The round (control) end should always be blue indicating that the enzyme is active and has hydrolyzed the substrate. The sample end will also be blue if no anticholinesterase agents are present. However, if these agents are present, the enzyme will be inhibited and no blue color will be observed.

This reagent system is now being incorporated into a new chemical agent detector kit which is presently under development to replace the M15A2 and M18A2 kits.[2,3] One of the major considerations in the development of this kit is the simplification of the item. Several methods of exposing the detector ticket to the suspect atmosphere are being investigated to determine their usefulness in terms of human factors and sensitivity. This study was initiated on the enzyme system because it is a vital component of the kit and is the one which is required to detect the lowest concentration of toxic material. All existing detector kits utilize a mechanical pump of some type to impact the air sample on a surface as in the enzyme system in M15/M18 type kits or to draw air through the sample as with the detector tubes in the M15/M18 and most foreign and industrial kits. In attempting to simplify the kit, one approach considered was to eliminate the mechanical pump and simply place the detector in the suspect atmosphere – a static exposure. The detector could be hand waved in the suspect atmosphere. In order to evaluate these methods of sampling, a study was conducted using GB concentrations ranging from 0.003 to 0.10 μg/l as described in this report. For comparison, the detectors were also exposed by impaction and pullthrough procedures. In addition, a test was conducted with a special adapter and a pump in an attempt to detect very low concentrations (0.003 μg/l) as had been reported by the Canadians.[4]

The Q5, toxic gas, single- and double-dilution apparatus,[5] with some adjustments, was used to produce the low concentrations of GB.[6-8]

II. EXPERIMENTATION.

A. Reagents.

1. Indole Solution. Weigh 0.2 gm of indole, place in a 25-ml volumetric flask, and dilute to the mark with acetone. Keep refrigerated. Make fresh every 2 days.

2. Sodium Pyrophosphate Peroxide (SPP). Weigh 0.5 gm of SPP, place in a 25-ml volumetric flask, and dilute to the mark with deionized water. Keep refrigerated. Make fresh every 2 days.

3. Substrate. Weigh 25 mg of 2,6-dichloroindophenyl acetate, place in a 25-ml volumetric flask, and dilute to the mark with ligroin (density, 0.69 to 71 at 20°C).

4. Buffer. Tris(hydroxymethyl)aminomethane, 0.05 M, adjusted to pH 8.0.

5. Isopropanol. Deionized water solution, 1:1.

6. Stock GB. 100 μg/ml in 1:1 isopropanol-deionized water solution.

B. Apparatus.

1. Generator.

The Q5 toxic single-dilution and/or double-dilution generator was assembled (figure 1) with the following modifications: (a) the equilibrator (agent pot) was partially submersed in a cold water bath which rested on a thermoelectric cold plate connected to an on-off thermostatic temperature control unit – a modification required to maintain constant temperature and to assure constant agent concentration; and (b) a test chamber was connected to the reservoir of the dilution generator to provide ample room for enzyme ticket exposure by methods described later in this report.

2. Fluorometer.

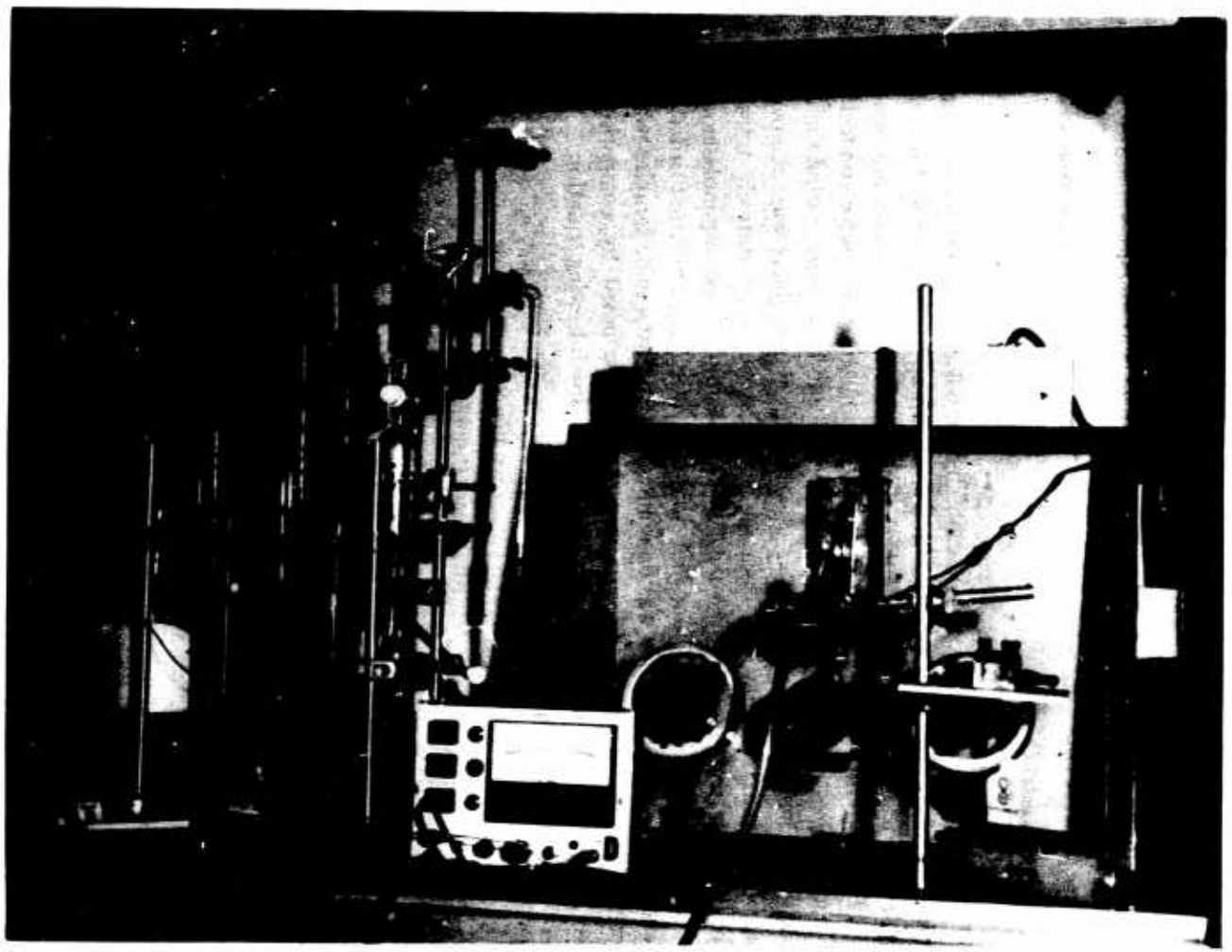
A Turner fluorometer, Model 111, with the following filters was utilized: primary filter, dark blue – 7-60; secondary filters: light blue – No. 4308, yellow – No. 2289; and Wratten ND filter, 1.00.

3. Bausch and Lomb Recorder.

A Bausch and Lomb VOM7 recorder was connected to the Turner fluorometer to record the fluorescence intensities.

4. Enzyme Tickets.

Enzyme tickets were made by the Mine Safety Appliance Company, Pittsburgh, Pennsylvania, to the specifications (MIL-D-51083C) of the M15A2 and M18A2 detector kits.



A. Q5 single-dilution generator
B. Test chamber
C. Thermoelectric cold plate
D. Thermostatic on-off temperature control

Figure 1. Q5 Single-Dilution Apparatus Connected to Test Chamber

C. Procedure.

1. Preparation of Standard Curve.

A standard curve was obtained by diluting appropriate aliquots of the GB stock solution to 23 ml with 1:1 isopropanol-deionized water solution. One milliliter each of indole and SPP was then added and the maximum fluorescence was read within 2 to 4 minutes. The results were corrected for the blank determinations.[6]

2. Analysis.

The Q5 generator was set up and calibrated for GB. Flow inside the test chamber used for the exposure of the enzyme tickets varied from 20 l/min at 0.02 μg/l to 40 l/min at 0.10 μg/l using the single-dilution apparatus and 8 l/min at 0.003 μg/l with the double-dilution apparatus. Two bubblers, each containing 5 ml of 1:1 isopropanol-deionized water, were connected in series between the test chamber and the vacuum line. The vacuum and timer were started simultaneously. At appropriate time intervals, the vacuum line was shut off, the bubblers were removed, and the sampling port was closed. The solutions from the bubblers were quantitatively transferred to a glass-stoppered graduate cylinder and diluted to 23 ml with 1:1 isopropanol-deionized water solution. One milliliter each of indole and SPP solutions was added (in that order) and mixed thoroughly, and maximum fluorescence was determined within 2 to 4 min. Samples were corrected for blank determination, and agent concentration[6,7] was determined by comparison with the standard curve. The sample concentration was determined by the following equation:

$$\text{Conc GB } (\mu g/l) = \frac{\text{Net Turner reading} \times \text{agent factor}}{\text{Flow rate (l/min)} \times \text{time (min)}}$$

3. Enzyme Ticket.

Tickets were wetted with two drops of buffer before exposure to agent by one of the following methods:

a. Pullthrough (figure 2) – The enzyme ticket was placed between two round Teflon-coated brass disc holders and inserted between the test chamber and the vacuum line. The vacuum and timer were started simultaneously. GB vapor was drawn through the ticket.

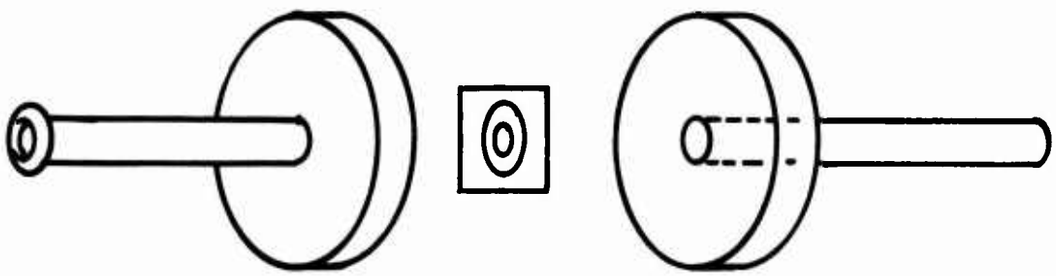


Figure 2. Holder for Pullthrough Exposure

b. Impaction (figure 3) – The enzyme ticket was placed in Lucite block holders which were inserted between the vacuum line and the test chamber. The vacuum and timer were started simultaneously. GB vapor was impacted on the ticket.

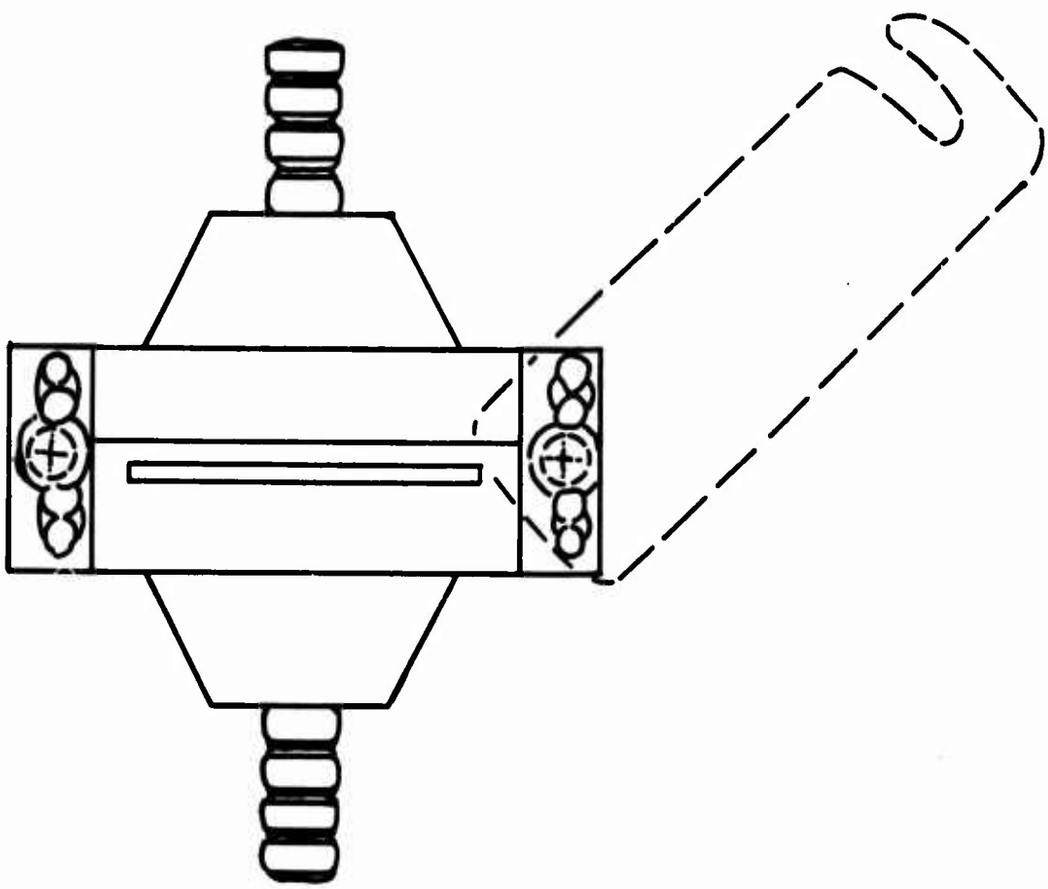


Figure 3. Holder for Impaction Exposure

c. Diffusion, static (figures 4 and 5) – The enzyme ticket was hung from an alligator clip which rested on an arm remaining stationary inside the test chamber.

d. Diffusion, waving (figures 4 and 5) – The enzyme ticket was hung from an alligator clip which rested on an arm inside the test chamber. The arm's movement was mechanically controlled by a switch mounted outside the test chamber. The arm's speed was 110 to 120 rpm.

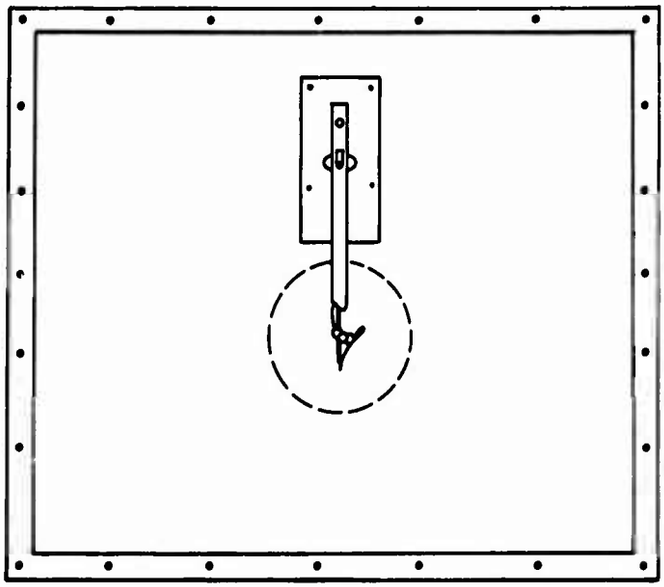


Figure 4. Front View of Agent Box Showing Arm, Clip Holder, and Opening for Introducing Sample

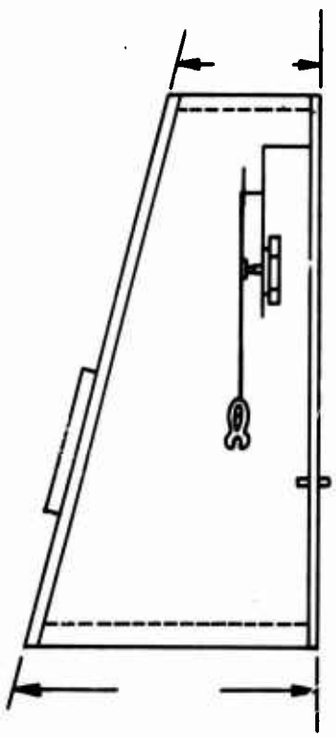


Figure 5. Side View of Agent Box Showing Arm and Clip Holder

After exposure for the desired time, the ticket was removed from the test chamber and treated as follows: one drop of tris buffer was added to the ticket, placed in a plastic envelope, and kneaded until wetted (approximately 30 sec). Then two drops of substrate were added and kneaded for 30 sec. After a total elapsed time of 2 min, the color on the disc was noted.

The pump and adapter described in the Canadian report are shown in figure 6. The enzyme ticket is placed in the adapter, and the end is tightened on the threaded end to form an O-ring seal around the enzyme impregnated paper. The pump is then used to draw suspect air through the paper by taking 40 strokes (100 ml/stroke). The number of strokes to be taken was determined by the agent concentration.

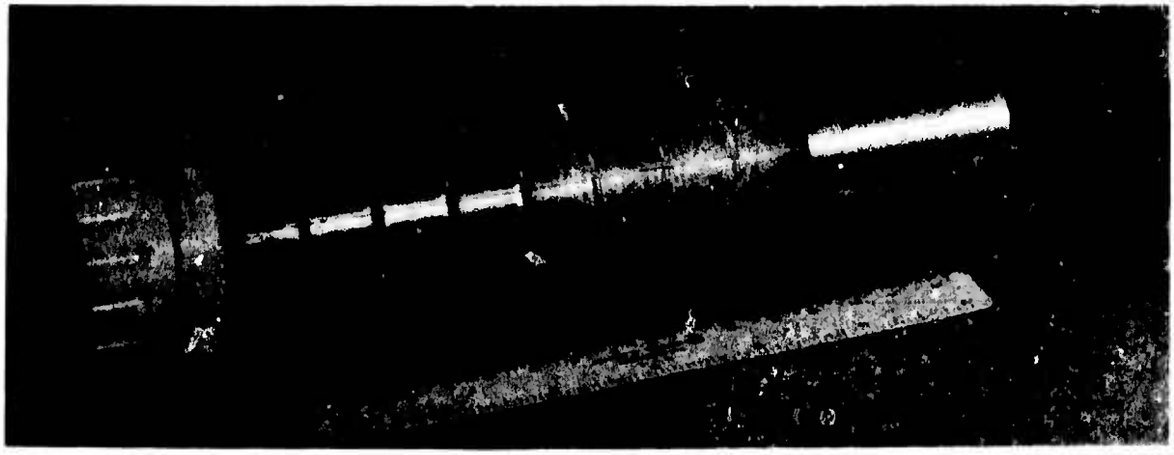


Figure 6. Pump and Adapter for Low Concentration of Toxic Agents

III. DISCUSSION AND RESULTS.

Preliminary experiments were conducted to determine the time of exposure for inhibition of enzyme tickets. The GB generator was stabilized at 0.02 μg/l. The ticket was then exposed for 1 min. If color appeared, the test was repeated, increasing the exposure time to 2 min, 3 min, 4 min, etc. until complete inhibition was obtained on 10 tickets in succession. This time was recorded as maximum exposure time. Once the maximum exposure time was determined, this time was divided into four intervals. For example, if 6 min were required for inhibition of 10 tickets, tests were repeated using 10 tickets at each time interval, 4.5, 3.0, and 1.5 min. This pattern was repeated for each concentration and each method of exposure until a minimum time allowing complete inhibition was established. This time was recorded as minimum exposure time.

Table I represents over 1,000 tests and shows the minimum time requirement at which compelte inhibition is assured. The agent detection by pullthrough and impaction was more rapid than detection by waving or static exposure. During these tests, the room temperature ranged from

75° to 85°F. The humidity in the test chamber was approximately 20% to 30% due to the drying of the house air used in dilution.

Table I. Detection of GB with Enzyme Tickets

Type test	GB concentration (μg/l)				
	0.02	0.04	0.06	0.08	0.10
	Time for positive test				
	min				
Impaction	3	2	1	0.8	0.6
Pullthrough	3	1.6	0.8	0.8	0.6
Diffusion, static	8	6	4	3.6	3.2
Diffusion, waving	6	4	3	2.4	2.0

A four-column, one-way analysis of variance was conducted to determine whether or not the time for a positive test showed a significant difference between the methods of exposure.

Table II. Analysis of Variance

Source of variance	Sum of squares	Degrees of freedom	Mean square	Ratio
Among columns	40.582	3	13.527	6.534
Within columns	33.120	16	2.070	
Total	73.702	19		

$F_{.01} = 5.29 < 6.534$

$F_{.05} = 3.24 < 6.534$

Analysis of variance showed that at the 95% and 99% confidence level a significant difference exists in the method of exposure.

Figures 7 through 10 show regression equation for minimum time requirements versus concentrations.

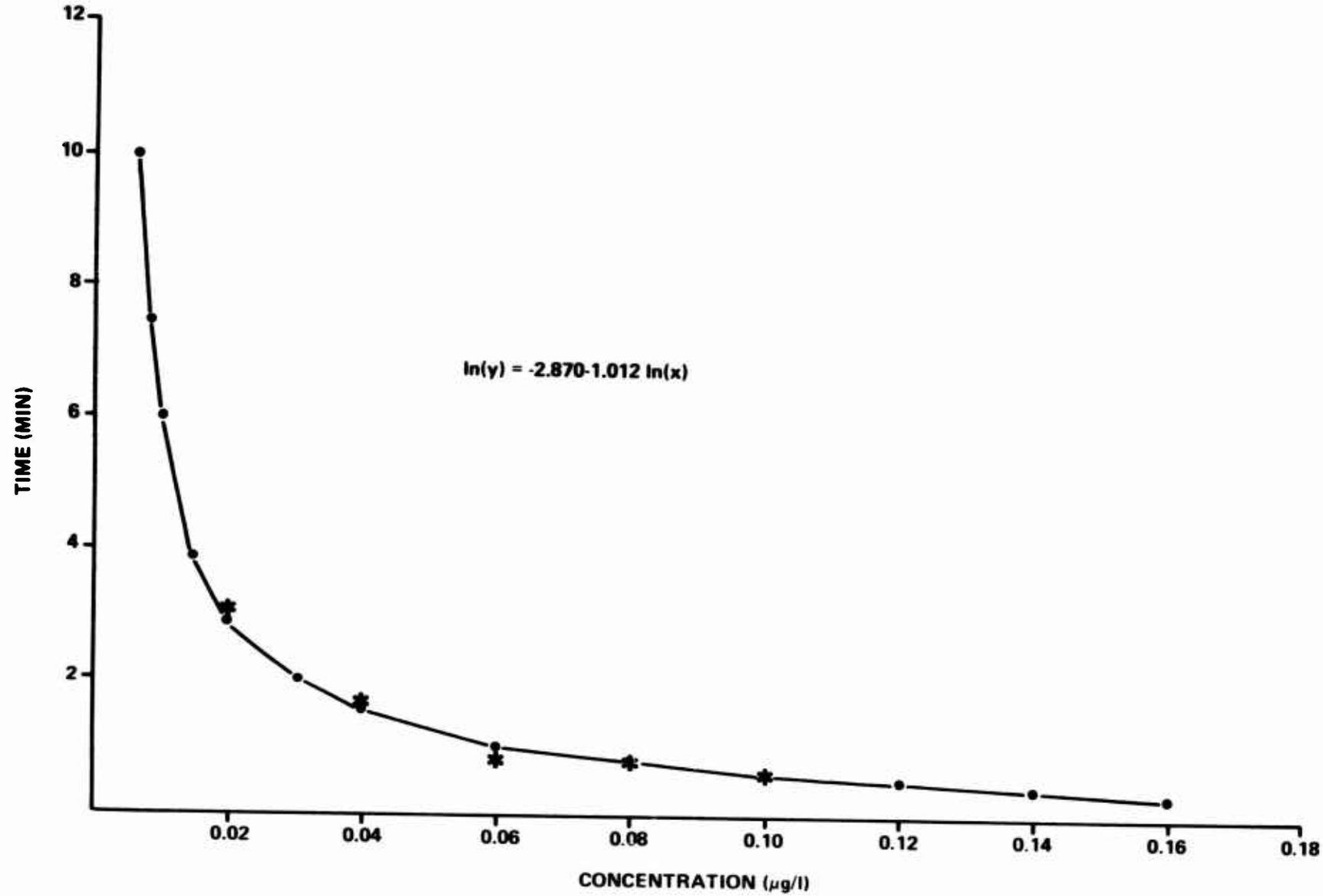


Figure 7. Enzyme Ticket Exposure to GB by Pullthrough Method

(Observed data)

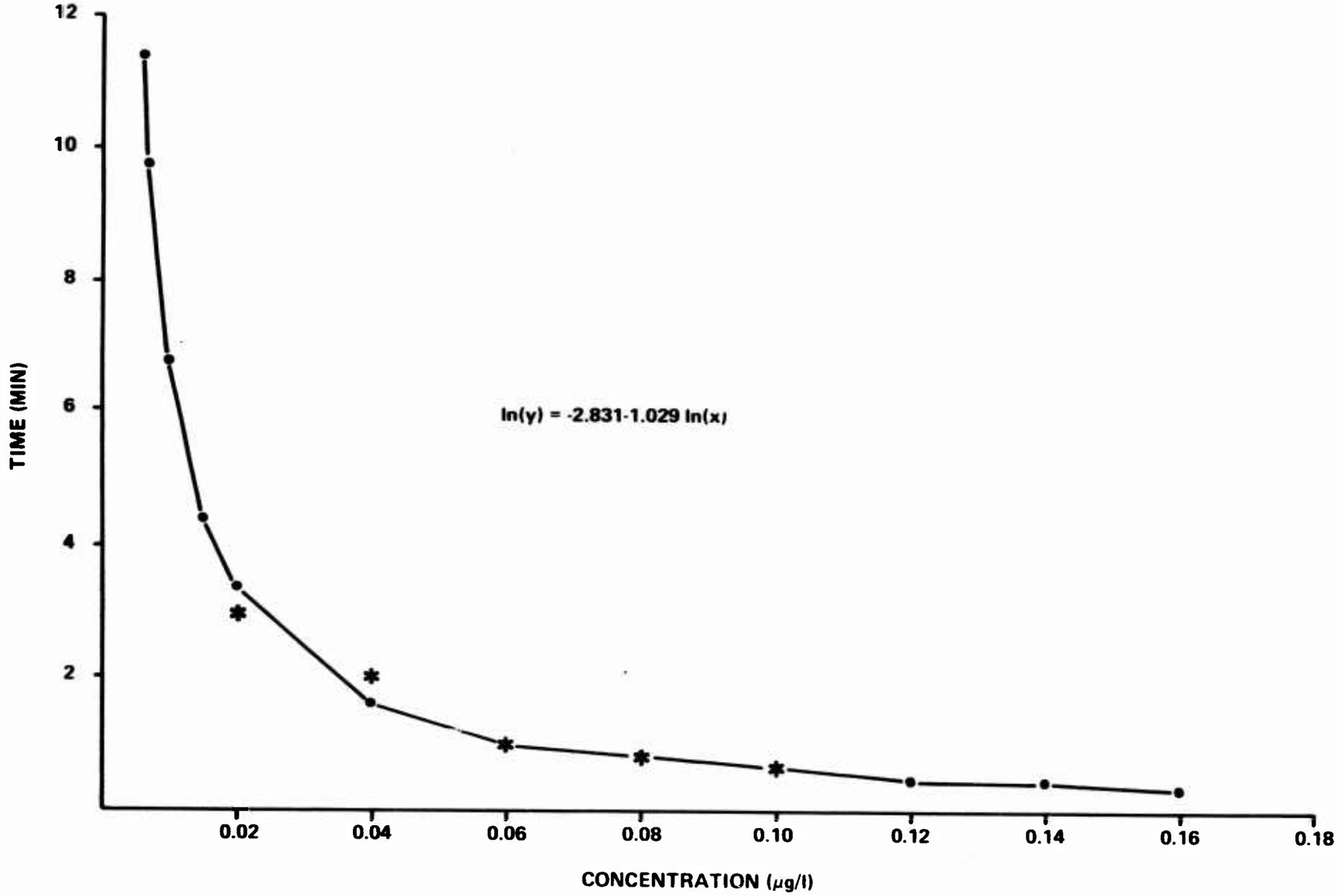


Figure 8. Enzyme Ticket Exposure to GB by Impaction Method

(Observed data)

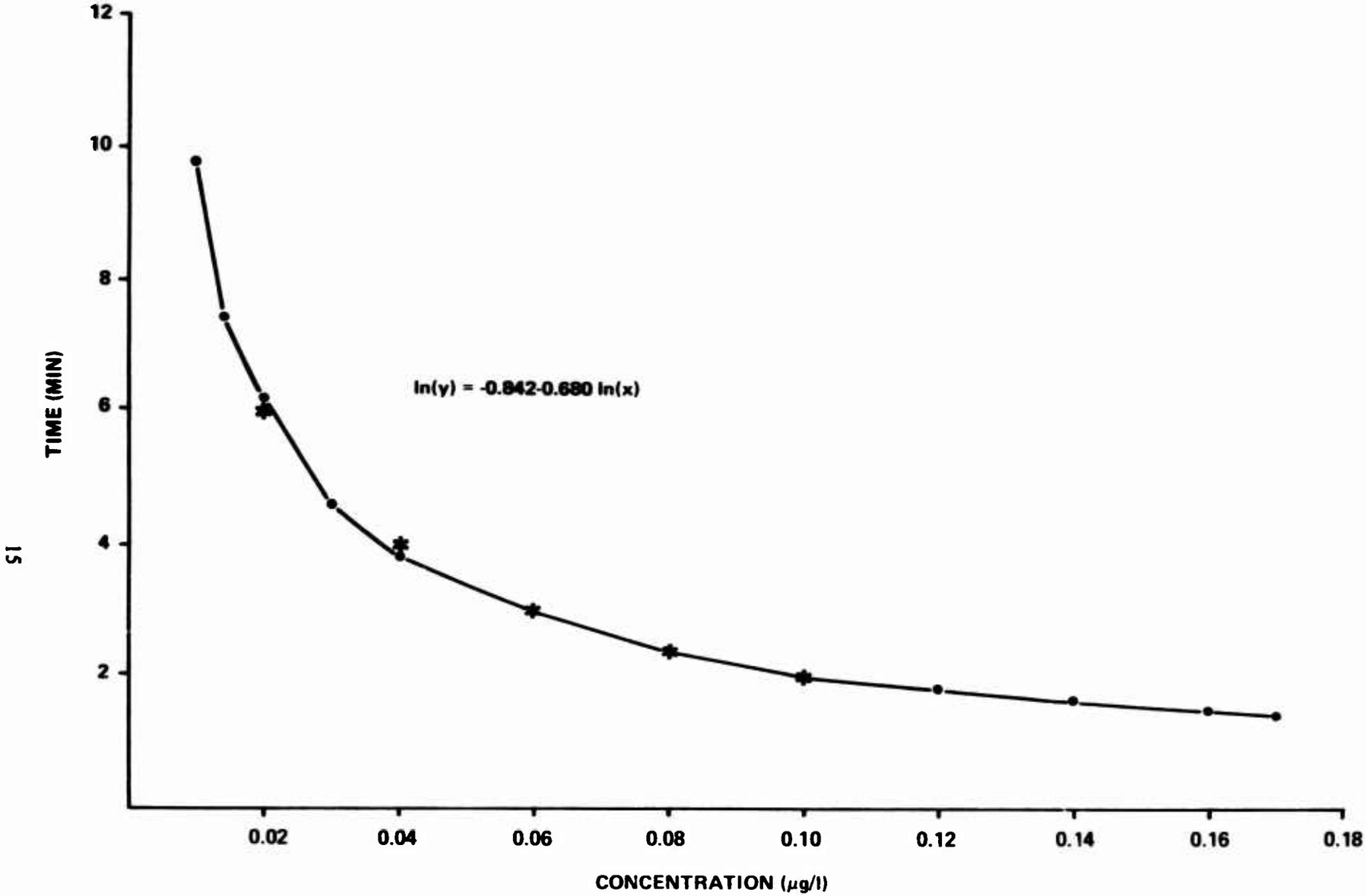


Figure 9. Enzyme Ticket Exposure to GB by Diffusion-Waving Method

(Observed data)

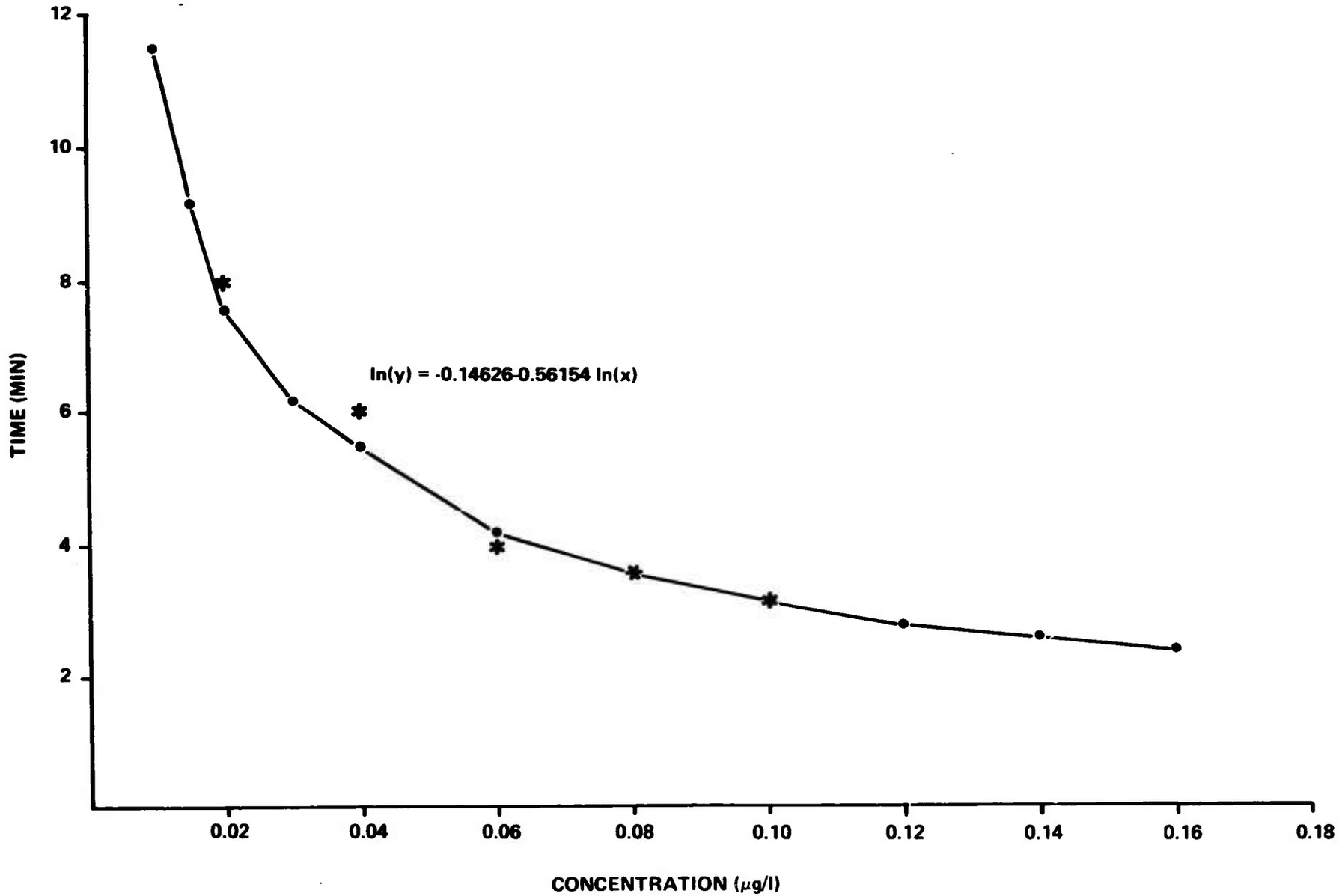


Figure 10. Enzyme Ticket Exposure to GB by Diffusion-Static Method

(Observed data)

In addition, in order to determine response at lower agent concentration, approximately 350 tests were conducted at 0.003, 0.007, and 0.01 μg/l using the double-dilution apparatus and the various techniques of ticket exposure. At the two lower concentrations, rewetting of the enzyme ticket with the tris buffer was necessary because of drying of the ticket due to longer exposure time (table III). The low relative humidity in the test chamber was undoubtedly a factor in the drying of the ticket and the need for rewetting. At higher humidities, it is assumed that the rewetting would be required at less frequent intervals, if needed at all. Although exposure times become lengthy, agent can still be detected by static exposure.

Table III. Detection of Very Low Concentrations of GB

Type of exposure	GB concentration (μg/l)		
	0.003[a]	0.007[b]	0.010
	Exposure time for positive test		
	min		
Pullthrough	–[c]	10	3
Impaction	–[c]	9	3
Waving	30	15	12
Static	90	30	24

[a] All tickets were rewetted three times during exposure to agent.
[b] All tickets were rewetted once during exposure to agent.
[c] Insufficient data.

The Canadian pump and adapter were able to detect 0.003 μg/l using 40 strokes. This is consistent with data reported by the Canadians. The sampling time is approximately 2 min.

Detection of agent in the field by pullthrough or impaction techniques requires a mechanical device to move the air. The diffusion methods, waving or static, do not require this equipment in the field, and it is possible to detect very low concentrations of agent if sufficient exposure time is used. Troops use the devices to determine whether it is safe to unmask and, hence, are protected while conducting the tests. Therefore, time is of less importance than in the case of an automatic alarm or warning device where the user is unprotected.

IV. CONCLUSIONS.

It can be concluded from analysis of the data that the M15/M18-type enzyme tickets met the requirements of the chemical agent detection kit to detect 0.03 ± 0.02 μg of GB/l in less than 10 min by static and waving exposures. The data show that GB vapor can be detected at lower concentrations and in less time with the pullthrough and impaction methods than in the kit requirement. All four methods fall within the kit requirement time at a concentration of 0.02 μg/l.

The pullthrough and impaction methods would require mechanical sampling equipment for field use. However, the diffusion waving or static method could be employed without special equipment. This gives additional support to the decision to simplify the design of the kit by eliminating mechanical devices for sampling suspect atmosphere.

In the event that very low concentrations of nerve agent must be detected (e.g., 0.003 μg/l), it can be accomplished by static exposure for long periods, up to 90 min, but requires periodic rewetting. These concentrations can be sampled in shorter periods, 3 min, with a mechanical pump and a special adapter to hold the ticket.

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3. The point of contact is Adana Eilo, ECBC Security Specialist, (410) 436-2063 or adana.l.eilo.civ@mail.mil.

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NANCY CARTER
Acting Security and Surety Manager

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